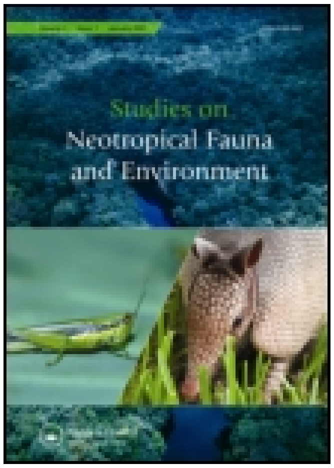

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ORIGINAL ARTICLE

Diversity of leafhoppers (Hemiptera: Cicadellidae) in experimental rice lots and associated weeds in Buenos Aires province, Argentina

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We identified the species of Cicadellidae associated with rice fields from an agricultural experimental station of La Plata city, Argentina, and explored the diversity pattern of the leafhopper assemblage. Insects were obtained from rice and surrounding weeds between 2006 and 2010. *Syncharina argentina* (Berg) and *Agalliana ensigera* Oman were the most abundant species in rice and weeds, respectively. Diversity (H′), heterogeneity (D) and evenness (J) indices were estimated. There was a significant effect of crop cycle (2006 to 2010) and host plant (rice and weeds) on H′ and D, with the interaction between them being not significant. Evenness was similar in rice lots and weeds, but differed between crop cycles. The pattern of abundance of species within rice lots and weeds followed the logarithmic model. Possible explanations for this pattern are discussed. The importance of the presence of some species as vectors of plant pathogens is highlighted.

Keywords: leafhoppers; rice field; biodiversity; range-abundance models; Argentina

Introduction

Cicadellidae (leafhoppers) is an important insect taxon from the agricultural point of view. It includes vectors of pathogens that cause diseases in crops with high economic significance (Maramorosch & Harris 1979). Nearly 70% of insect species transmitting plant disease agents are from this family (Nielson 1985). Leafhoppers also cause mechanical damage to plants during feeding and oviposition (Nault & Ammar 1989; Backus et al. 2005).

Among the cultivated plants affected by leafhoppers, rice (*Oryza sativa* L.) is one of the most important because of its high nutritional value. It is the main food of two-thirds of the world's population, and the basic livelihood for more than 30 countries in Asia, Africa, Latin America and the Caribbean (Wilson & Claridge 1991). The Argentinean provinces Entre Ríos and Corrientes account for around 90% of the country's production of rice (Fundación Proarroz 2013).

In Argentina, knowledge about Auchenorrhyncha affecting rice and the diseases they transmit is scarce. A survey of Cicadellidae from rice fields in Corrientes province revealed the presence of eight species (Remes Lenicov & Tesón 1985). In Entre Ríos province the most abundant species found was *Syncharina argentina* (Berg 1879) (Paradell et al. 2006). A literature review of *Syncharina* Young, which is widespread in South America, revealed that two of the four species in this genus, namely *S. punctatissima* (Signoret 1854) and *S. argentina*, are associated with rice and spontaneous vegetation (Remes Lenicov, Mariani et al. 2006; Paradell et al. 2011). Another important species found on rice is *Agalliana ensigera* Oman 1934 (Paradell et al. 2006). It has a Neotropical distribution and occurs in northern and central Argentina (Christensen 1942; Remes Lenicov 1982). It is also often registered on other horticultural, cereal and forage crops such as maize (Paradell et al. 2001), alfalfa (Meneguzzi 2008), garlic (Catalano 2011) and hairy vetch (Paradell et al. 2014). This leafhopper is the most important vector of "Argentine Curly Top" disease of sugar beet (Fawcett 1927) and "Brazilian Curly Top" of tomato (Costa 1952) in South America. In addition, this species was considered as a potential vector of Ar AWB "Witches broom" in alfalfa and 16 SrIII (X-disease) "Garlic decline" disease by Meneguzzi (2008) and Catalano (2011), respectively.

Results about the diversity of Delphacidae and Cixiidae (Fulgoromorpha) associated with rice were published recently (Remes Lenicov et al. 2014), but studies on leafhoppers on rice in Argentina are scarce. In order to systematically survey the species associated with rice crops, we conducted a sampling program designed to assess the number of species of

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Cicadellidae present, and to estimate their diversity, heterogeneity and evenness.

Leafhoppers may exploit plants for different resources, such as food, oviposition sites and shelter (Harris 1979; Nault & Ammar 1989). After settlement, species can compose an assemblage [defined as a part of the community whose members are phylogenetically related (Faulth et al. 1996)] resulting from the interactions between the component species and the constraints imposed by the habitat. The assemblage may assume a particular model of abundance distribution, which is a mathematical description of abundance data. Species may also have the possibility of colonizing contiguous but distinct habitats. The outcome of this would be a different species abundance model in each relatively homogeneous part of the habitat. The description of such abundance patterns is as important as the measurement of diversity in a community (Tokeshi 1993). In order to ascertain patterns of the structure of the assemblage of Cicadellidae within the agronomic ecosystem under study, we explored the degree of adjustment of the theoretical distribution of species, based on the frequency of individuals, to the actual observed distribution. Our interest was to characterize the α-diversity pattern of leafhoppers in the rice culture and to assess if this pattern differs amongst the sub-systems formed by rice plots and surrounding weeds. Weeds may be another source of biological harm for the cultivation of rice, as they can reduce the maximum potential yield (Lovato Echeverría et al. 2013). Therefore, we explored differences among species present, and their diversity patterns in both sub-systems of the rice field.

Material and methods

Sampling site

Collections were performed in a rice field at the Agricultural Experimental Station "Julio Hirschhorn" of the Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires province, Argentina (34°56′57″ S, 57°58′22″ W). The field is a rectangle of 0.6 ha encompassing 48 plots of approximately 11 × 11 m each. The boundaries of the field are non-cultivated strips with spontaneous vegetation (weeds), which separate the rice field from other crops. Sampling was performed along four cropping seasons from 2006 to 2010. Each rice crop cycle lasted from November (seeding) to April (harvest). Samples were obtained from both the rice plots and the strips with weeds. Weeds surrounding the culture were identified according to Cabrera & Zardini (1978).

Cicadellid sampling

Sampling started on December 2006 and ended on March 2010. Each year, collections began after rice plants had more than four leaves. Insects were collected weekly with a trawl sweep net (40 cm in diameter). Fifty successive strokes per sampling date were performed on six randomly selected rice plots (total: 300 sweeps per week). Another 50 sweeps were carried out on each strip (front, right, left and back sides) of the surrounding weeds (total: 200 sweeps per week). Specimens were dissected under a stereoscopic microscope using conventional techniques (Young 1977) in order to be identified. Identification followed Linnavuori (1959), Paradell (1995a, 1995b), Remes Lenicov & Tesón (1985) and Wilson & Claridge (1991). Individuals were deposited and compared with reference specimens hosted at the collection of Museo de La Plata, Argentina.

Data analysis

Samples were assorted according to the portion of the field where they were obtained (rice plots or the surrounding strips with weeds). Numbers of individuals were summed up for each sampling subset (rice and weeds hereafter) within each species, considering the total caught from all samples obtained between 2006 and 2010. To estimate the local diversity of leafhoppers and its temporal fluctuation, we calculated diversity, heterogeneity and evenness. Diversity was estimated with the Shannon–Wiener index as $H' = -\sum p_i \ln p_i$, where p_i is the proportion of the ith species in relation to the total number of species per sample. Heterogeneity was estimated with the Simpson index as $D = \sum p_i^2$. Evenness was estimated with the Shannon–Wiener index as $J' = H'/H'_{max}$, with $H'_{max} = \ln(S)$, where S is the species richness (Pielou 1969; Peet 1974; Krebs 1989).

Comparisons of diversity, heterogeneity and evenness among the four cropping cycles and host plants, and their interaction, were tested using two-way analysis of variance (ANOVA) on raw data. Data were not transformed because normality of H′, D and J′ was verified after applying the Kolmogorov–Smirnov goodness of fit procedure (Zar 1984). Variation between rice plots, or between strips with weeds surrounding plots, was included as the experimental error. Each sample consisting in 50 net strokes was considered as a replicate (six replicates on rice and four replicates on weeds per sampling date). *A posteriori* comparisons between significant effects was performed with Tukey's honest significance test with α =0.05 (Zar 1984).

Species of leafhoppers were arranged according to their abundance. Species lists and their abundances differed between host plants; thus, two lists were ranked from the most to the less abundant. After that, each observed frequency distribution was adjusted to the expected distribution, according to the following models: geometric series, log-normal distribution, logarithmic series and the broken stick model. Methods for obtaining expected distributions followed procedures described in Krebs (1989) and Magurran (2004). Differences between expected and observed abundance curves were calculated using χ^2 tests for the H_0 of no difference between them, with α =0.05.

Results

A total of 4113 individuals of Cicadellidae were caught, 684 (17%) on rice and 3429 (83%) on weeds. We identified 39 species, belonging to 29 genera. We recorded 25 species in rice plots and 38 in the surrounding natural vegetation (Table 1). Thus, both species richness and abundance of individuals were higher in weeds than in rice. The abundance plot for all species shows a faunal distribution with a single species with > 1000 individuals, 17% of species with > 100 individuals and 38% with less than 10 individuals (Figure 1). The most abundant species on rice were *S. argentina* (n = 377), *Haldorus sexpunctatus* (Berg) (n = 59) and *A. ensigera* (n = 55), and on weeds, *A. ensigera* (n = 1016), *S. punctatissima* (n = 628) and *Exitianus obscurinervis* (Stål) (n = 403). The following taxa were recorded as singletons in rice: *Pawiloma victima* (Germar), and in weeds: *Tapajosa rubromarginata* (Signoret), *Mendozellus asunctia* Cheng, and *Unerus* sp.

ANOVA showed a significant effect of crop cycle and host plant (rice and weeds) on both diversity estimated with Shannon–Wiener's H′ (Figure 2) and heterogeneity estimated with Simpson's D (Figure 3), with the interactions between them being not significant (Table 2). Evenness estimated with J′ was similar for rice plots and weeds, but it differed significantly among crop cycles (Figure 4); the interaction term (crop cycle × host plant) had a not significant effect on evenness (Table 2). Diversity and heterogeneity were highest in the first (2006–2007) and last crop cycle (2009–2010), and lowest in the second (2007–2008) and third (2008–2009); diversity and heterogeneity were also significantly higher in weeds than in rice (Figures 2, 3).

There were no significant differences (p > 0.05) between the observed distributions of leafhopper species sampled on rice and on weeds and the logarithmic series (Table 3). The geometric series, log-normal distribution and the broken stick model did not fit the observational data of species abundance on rice or on weeds (p < 0.05).

Discussion

Leafhopper diversity

The current study on an assemblage of Cicadellidae from temperate Argentina shows that rice fields can sustain a diverse community, even though they comprise habitats characterized in general by low complexity, compared to non-cropped landscapes. Despite this many leafhopper species can colonize rice plants. The majority of rice farming activities in Argentina take place in its northernmost latitudes, thus, a more diverse assemblage of leafhoppers can be expected than in La Plata. However, comparisons are not possible between the relative abundance of species associated with rice in the two latitudes because no information about the number of individuals per species was provided by Remes Lenicov & Tesón (1985). On the basis of presence/absence, four species sampled by us on rice, *Ciminius platensis* (Berg), *Hortensia similis* (Walker), *S. argentina* and *S. punctatissima*, were also recorded in Corrientes province (about 800 km distant from La Plata) (Remes Lenicov & Tesón 1985). Species richness of leafhoppers in La Plata was similar to richness found by Paradell et al. (2006) in Entre Ríos province. Moreover, they found that *S. argentina* was the most abundant species on rice and *A. ensigera* on weeds, which agrees with the present study.

Annual changes in species diversity and heterogeneity were recorded, and these changes were independent of the host plants, as revealed by the not significant interaction between the effects of crop cycle and host plant in the ANOVA. The *a posteriori* tests of ANOVA grouped together the means of H′ and D for the 2007–2008 and 2008–2009 periods, thus the influence of factors reducing diversity and heterogeneity during the two crop cycles was similar. Technical aspects of the cropping practice (rice strain, date of seeding and flooding of plots, phenology of rice) did not differ noticeably among crop cycles. Therefore, other factors such as weather variables and biological interactions may influence the set of species, but their impact remains as yet unknown.

Weeds sustained a richer and more varied leafhopper assemblage than rice. This was not unexpected because the spontaneous vegetation comprises more plant species than a monoculture, and consequently the availability of resources would be higher. Diversity in rice would be dependent on feeding or oviposition choice by insects; this would explain why fewer species were associated with rice than with

Table 1. Spectrum and abundance of species of Cicadellidae collected on rice plots and surrounding weed strips, at the Agricultural Experimental Station "Julio Hirschhorn", La Plata, Argentina during 2006–2010. Percentage of each taxon on rice and weeds is given in brackets.

	Abundance	
Species	Rice	Weeds
Agallinae		
Agalliana ensigera Oman	55 (8)	1016 (30)
Bergallia signata (Stal)	1 (< 1)	4 (< 1)
Cicadellinae		
Bucephalogonia xanthophis (Berg)	—	3 (< 1)
Ciminius platensis (Berg)	1 (< 1)	10 (< 1)
Draeculacephala sp.	4 (< 1)	2 (< 1)
Hortensia similis (Walker)	1 (< 1)	2 (< 1)
Pawiloma victima (Germar)	1 (< 1)	—
Plesiommata mollicella (Fowler)	1 (< 1)	48 (1)
Syncharina argentina (Berg)	377 (55)	73 (2)
Syncharina punctatissima (Signoret)	22 (3)	628 (18)
Tapajosa rubromarginata (Signoret)	—	1 (< 1)
Coelidiinae		
Coelidia sp.	—	4 (< 1)
Deltocephalinae		
Amplicephalus dubius (Linnavuori)	8 (1)	5 (< 1)
Amplicephalus faminoides Linnavuori	—	3 (< 1)
Amplicephalus marginellanus (Metcalf)	6 (< 1)	53 (2)
Amplicephalus simpliciusculus Linnavuori	4 (< 1)	70 (2)
Amplicephalus sp.	11 (2)	44 (1)
Atanus viridis Linnavuori	—	22 (1)
Atanus sp.	1 (< 1)	2 (< 1)
Balclutha lucida (Butler)	9 (1)	4 (< 1)
Balclutha rosea (Scott)	3 (< 1)	31 (1)
Chlorotettix fraterculus (Berg)	26 (4)	179 (5)
Chlorotettix neotropicus Jensen-Haarup	—	48 (1)
Chlorotettix sp.	4 (< 1)	1(< 1)
Clorindaia hecaloides Linnavuori	—	17 (< 1)
Exitianus obscurinervis (Stål)	36 (5)	403 (12)
Frequenamia spiniventris (Linnavuori)	—	2 (< 1)
Fusanus griseostriatus Linnavuori	—	6 (< 1)
Graminella puncticeps Linnavuori	30 (4)	21(< 1)
Haldorus sexpunctatus (Berg)	59 (9)	137 (4)
Mendozellus asunctia Cheng	—	1(< 1)
Spangbergiella vulnerata Signoret	3 (< 1)	107 (3)
Stirellus picinus (Berg)	3 (< 1)	8 (< 1)
Unerus sp.	—	1(< 1)
Gyponinae		
Curtara pagina De Long & Freytag	—	43 (1)
Reticana lineata Burmeister	—	14 (< 1)
Reticana sp.	—	3 (< 1)
Ledrinae		
Xerophloea viridis (Fabricius)	8 (1)	342 (10)
Typhlocybinae		
Empoasca curveola Oman	10 (1)	71 (2)
Total number of individuals	684	3429
Total number of species	25	38

weeds. Although there is a lack of studies concerning host preferences of Cicadellidae from Argentina, on the basis of previous experience, we observed that *S. argentina* can complete its development from egg to adult on rice plants (Barbara S. Defea, unpubl. data), and that in rice fields from other parts of the country its populations peak during the initial period of the culture (Remes Lenicov & Tesón 1985). In Entre Ríos province, *S. argentina* is the most abundant species (Paradell et al. 2006). This taxon, although not a feeding specialist, had a close association with rice and deserves further studies in the laboratory in

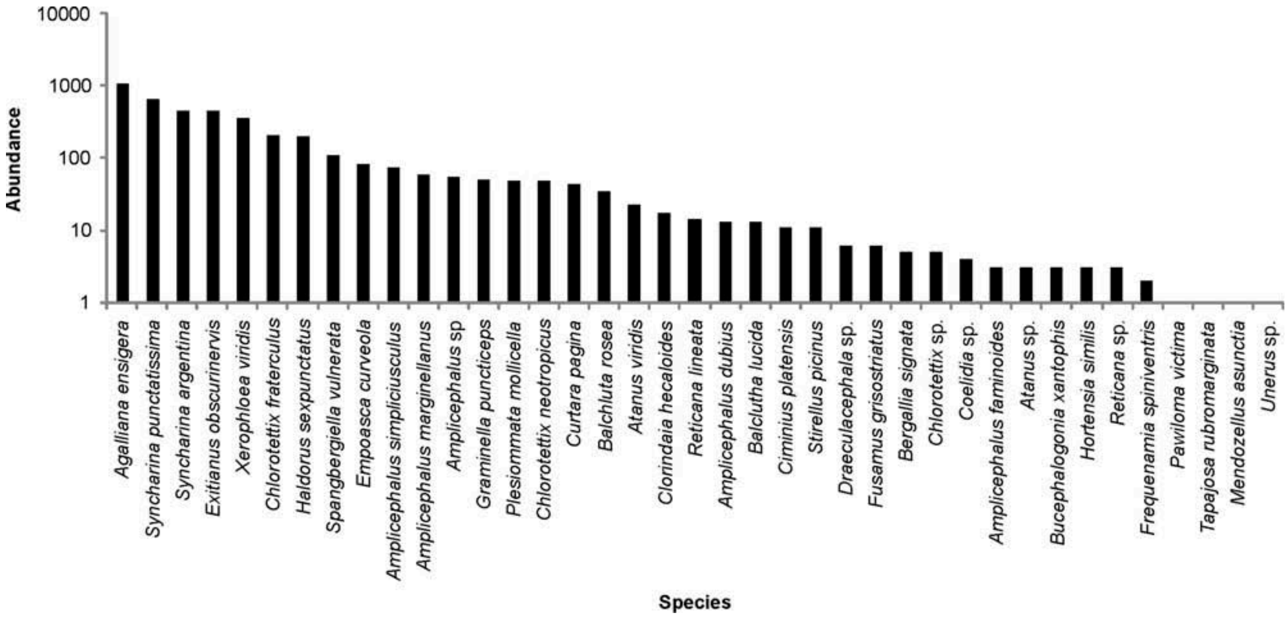

Figure 1. Ranking, according to abundance, of species of Cicadellidae collected at La Plata, Buenos Aires, Argentina, between 2006 and 2010.

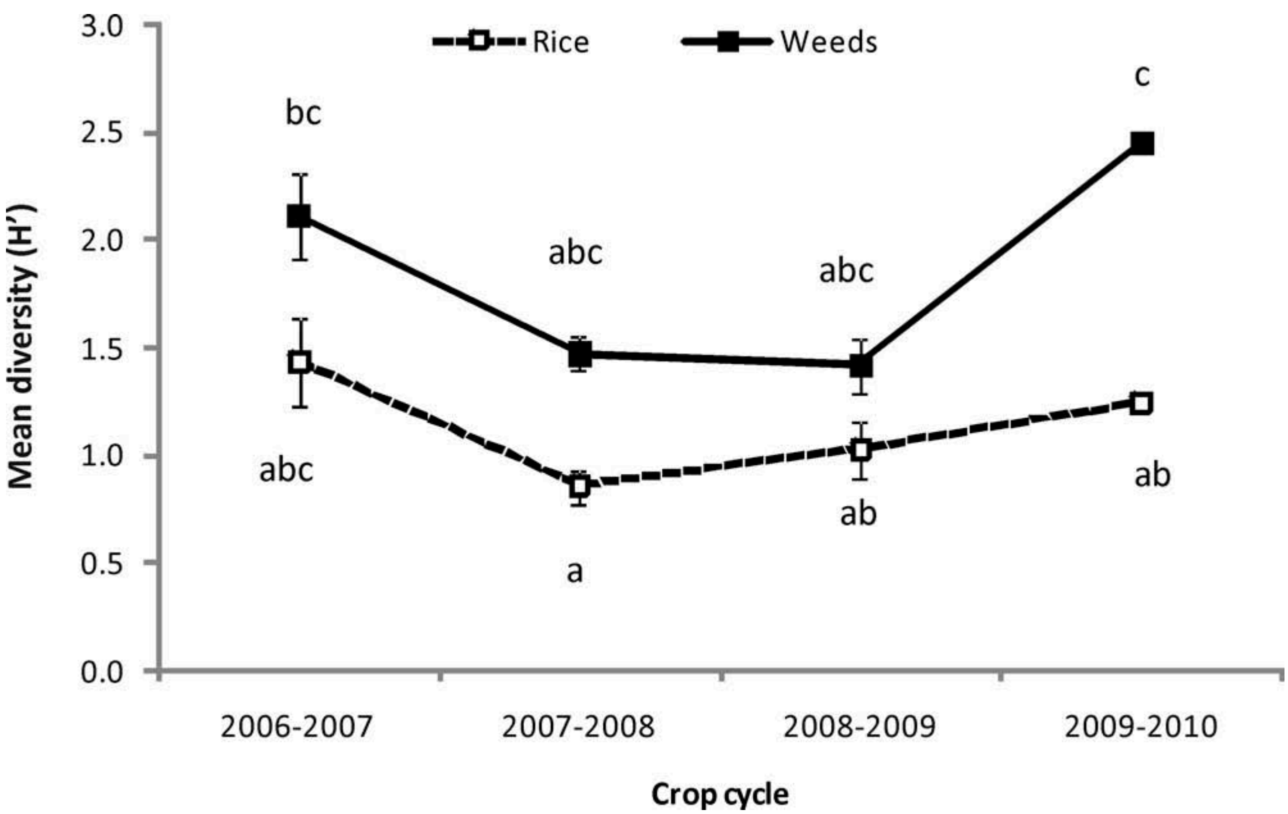

Figure 2. Mean diversity (H′) in the assemblage of Cicadellidae from La Plata, Buenos Aires, Argentina, collected along four crop cycles (2006 to 2010) on rice plots and surrounding weeds. Means followed by the same letter are not statistically different. Vertical bars denote ± SE.

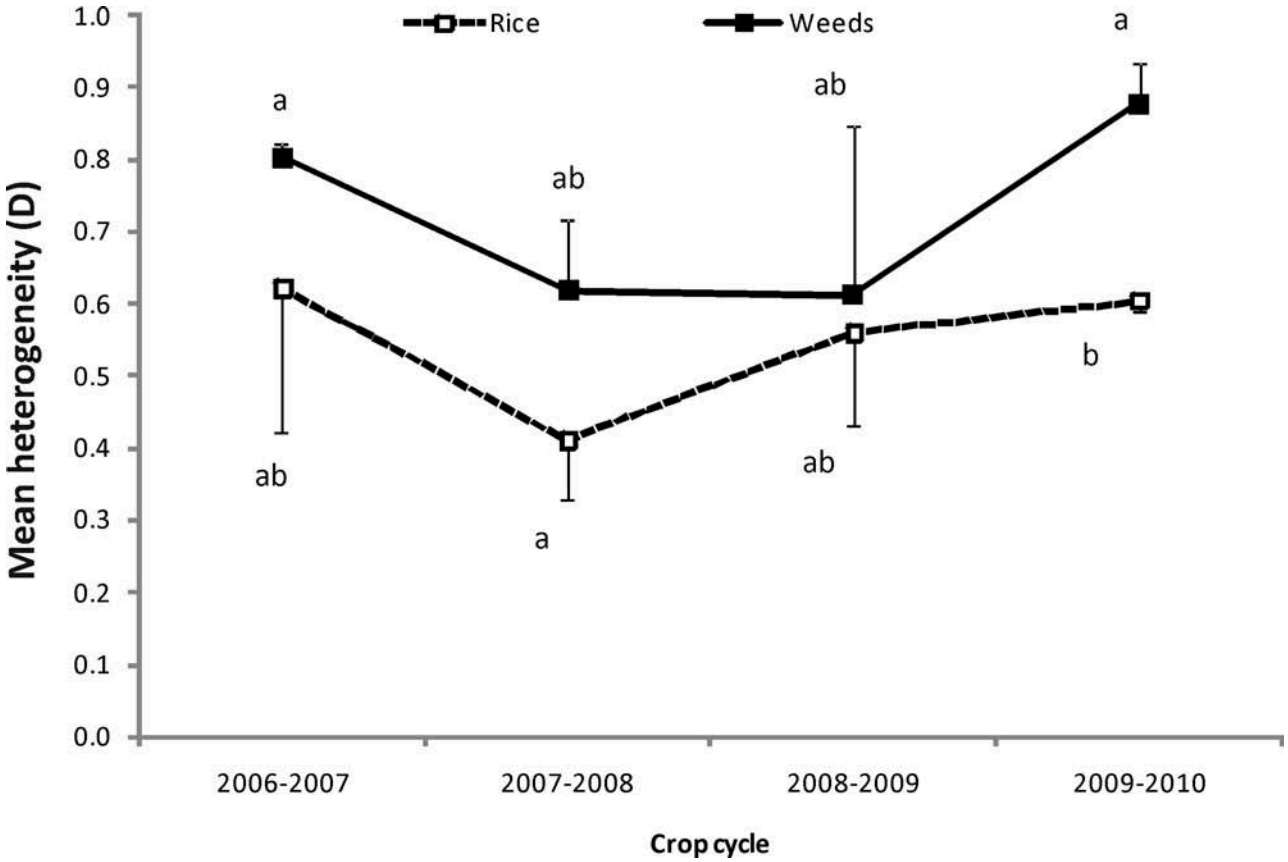

Figure 3. Mean heterogeneity (D) in the assemblage of Cicadellidae from La Plata, Buenos Aires, Argentina, collected along four crop cycles (2006 to 2010) on rice plots and surrounding weeds. Means followed by the same letter are not statistically different. Vertical bars denote + or – SE (for weeds and rice, respectively).

order to obtain a sound knowledge about its preference for rice as a host.

Evenness did not differ significantly between rice and weeds. In other words, the degree to which individuals are distributed in an equitable manner among species was preserved regardless of the host plants for which it was calculated. Diversity of herbivorous insects such as leafhoppers may be maintained through low competitive exclusion, which in turn allows the coexistence of many species (Siemann et al. 1998).

The differences in abundance and richness among cropping cycles may be attributed to variability in the density of weeds associated with the crop. During the first and last cropping cycles higher values for these parameters may be linked to higher resource availability for insect species. The abundance of spontaneous plants associated with rice can influence the spatial distribution of leafhoppers, which in turn may reflect a more diverse offer of refuges in alternative hosts (Decante et al. 2009), with an increase in the number of insect species on them.

Species abundance models

We determined that the assemblage of leafhoppers followed a pattern of species distribution that fitted the log-series model for the case of insects sampled on rice. In the logarithmic series (Fisher et al. 1943), a few factors determine the structure of the community, and there is high heterogeneity. This agrees with the idea that rice is the most important element shaping the assemblage and favoring heterogeneity, owing to the existence of one or few dominant species. This may be the case for *S. argentina*, a species whose abundance exceeded the abundance of all the other species together occurring on rice (377 versus 307 individuals). Samples from weeds also fitted the logarithmic model. We expected that the spontaneous vegetation would harbor a more diverse and even distribution of leafhoppers, thus following the broken stick model (MacArthur 1957). This hypothesis was not sustained by the results, because although diversity was higher in weeds than in rice, evenness did not depend on host plants. We observed that spontaneous plants were often

Table 2. Results from ANOVA with crop cycle (2006 to 2010) and host plant (rice and weeds) as main effects, and diversity (H′), heterogeneity (D) and evenness (J′) as response variables.

	H′			D			J′		
Effect	df	F	*p*	df	F	*p*	df	F	*p*
Crop cycle (1)	3	4.70	0.008	3	3.32	0.03	3	2.98	0.048
Host plant (2)	1	19.08	0.0001	1	9.26	0.005	1	0.02	0.89 NS
(1) × (2)	3	0.99	0.411 NS	3	0.56	0.64 NS	3	0.87	0.47 NS
Error	28			28			28		

NS: not significant.

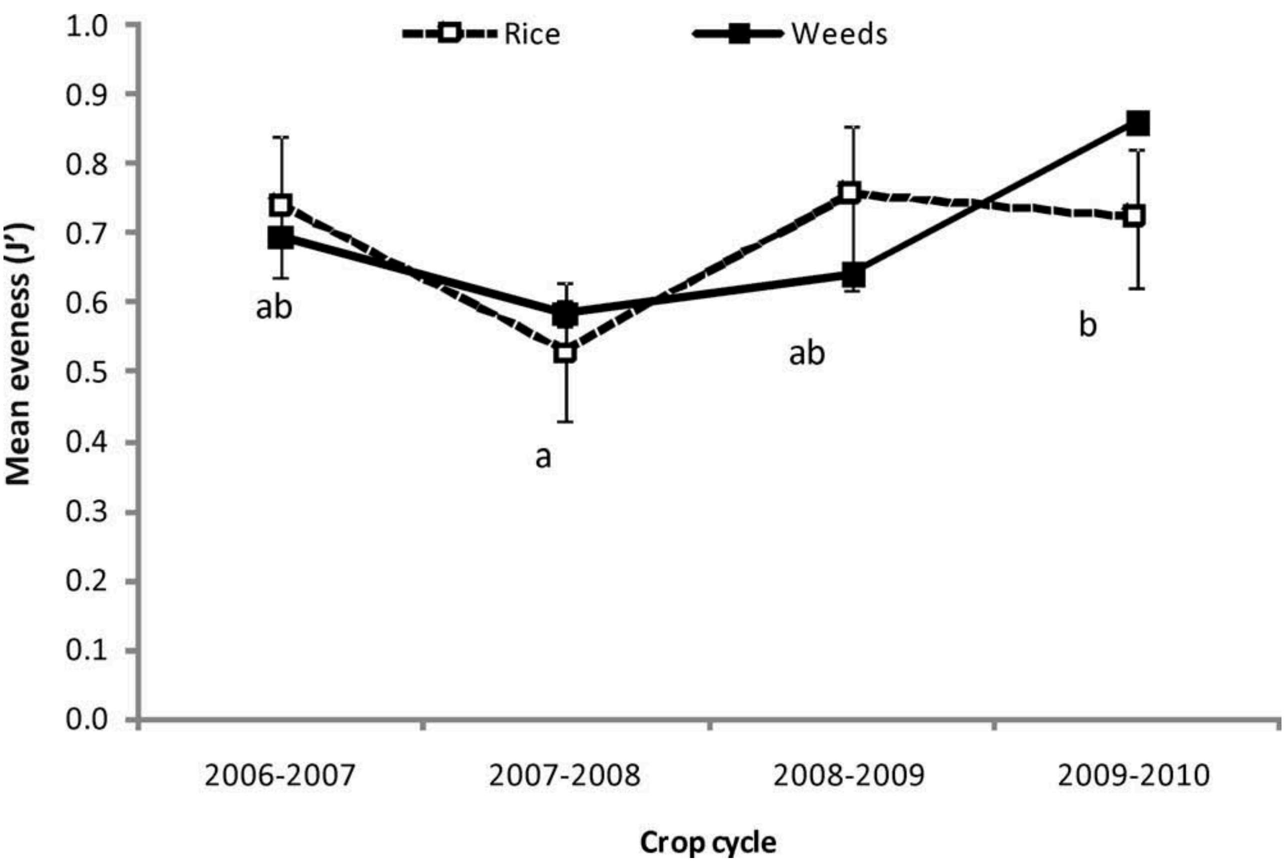

Figure 4. Mean evenness (J′) in the assemblage of Cicadellidae from La Plata, Buenos Aires, Argentina, collected between 2006 and 2010 on rice plots and surrounding weeds. Means for crop cycles followed by the same letter are not statistically different; as the effect of host plant was not significant, their means were not compared. Vertical bars denote ± SE.

Table 3. Values for χ^2 tests and *p*-values on H_0: no differences between observed and expected frequency distributions calculated by the geometric series, broken stick, logarithmic and log-normal models for abundance of species sampled on rice and weeds from rice plots from La Plata, Argentina, from 2006 to 2010.

	Rice			Weeds		
Model	χ^2	df	*p*	χ^2	df	*p*
Geometric	680.32	25	< 0.01	1267.28	33	< 0.01
Broken stick	92.27	8	< 0.01	280.36	9	< 0.01
Logarithmic	7.63	8	0.47	14.63	9	0.10
Log-normal	26.68	9	< 0.01	19.74	9	0.02

growing within rice plots, especially in periods when rice plants were artificially flooded. Consequently, the division between two distinct subsystems was sometimes lost, and the separation of the community would be somewhat artificial. However, at the population level, some species appeared to be attracted to specific parts of the agroecosystem, as was the case for *S. argentina* on rice.

Distribution of key species and economic importance

The geographical range of *S. argentina* comprises Brazil and Argentina (Young 1977). In the latter country it is mainly associated with cereal crops in the northern and central areas (Remes Lenicov & Tesón 1985; Tesón et al. 1986; Remes Lenicov & Virla 1993; Remes Lenicov, Paradell et al. 2006) and also with spontaneous vegetation (Paradell et al. 2011). It is important to highlight that some species [*Bucephalogonia xanthophis* (Berg), *E. obscurinervis*, *Graminella puncticeps* Linnavuori, *Stirellus picinus* (Berg)], associated with rice crops are vectors of pathogens in other parts of the world, mainly of other crops with high commercial importance (Linnavuori 1959; Young 1977; Wilson & Claridge 1991). *Agalliana ensigera*, the third most abundant species on rice, demands attention due to its role as a vector, as emphasized in previous studies (Fawcett 1927; Costa 1952; Paradell et al. 2006; Meneguzzi 2008; Catalano 2011; Paradell et al. 2014), a fact that reinforces the necessity of continuing basic studies about the transmission of pathogens that cause diseases in the region.

The lack of entomological studies focusing on the fauna that lives in rice fields and spontaneous vegetation in Argentina means that it is necessary that further studies are carried out on diversity and population dynamics of leafhoppers feeding on this crop. Monitoring should focus on the most important elements of biodiversity, such as vector species, since this may be better than spreading limited resources across larger surveys (Gaines et al. 1999). In this sense, our findings pointed to *S. argentina* as a key species in rice fields from Argentina.

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